DETAILED ACTION

This application is a 371 of PCT/US04/31224.

The amendment filed on August 26, 2011, has been entered.

Claims 1, 4-5, 8-11, 14-15 and 42 are pending and are under consideration.

Response to Arguments

Applicant's amendment and arguments filed on August 26, 2011, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 8-11, 14-15 and 42 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1, 8-11, 14-15 and 42 are drawn to a method of reducing the incorporation of non-standard amino acids into a heterologous protein in a microorganism by co-expressing in said microorganism said heterologous protein and a non-standard amino acid degrading protein that consists essentially of an *Escherichia coli* glutamate dehydrogenase having a leucine instead of lysine at the amino acid position that corresponds to amino acid position 92 of the wild-type *E. coli* glutamate dehydrogenase. It is noted that MPEP 2111.01 states that "[d]uring examination, the claims must be interpreted as broadly as their terms reasonably allow." In this case, the term "having" in transitional phrases does not create a presumption that the body of the claim is closed (See MPEP 2111.03). Therefore, a mutant of a wild-type *Escherichia coli* glutamate dehydrogenase having a leucine instead of lysine at the amino acid position that corresponds to amino acid position 92 of said wild-type glutamate dehydrogenase, wherein said mutant has non-standard amino acid degrading activity, is not limited to only the substitution at position 92. While the mutant comprises the recited substitution, the same mutant can comprise any amino acids in any other positions. Since the claims encompass a method of using any or all mutants of a glutamate dehydrogenase from *E. coli* comprising a leucine residue at position 92 and any other amino acids at any other position, the claims are drawn to a method of using a genus of glutamate dehydrogenase having non-standard amino acid degrading activity, but having unknown structure.

In *University of Calfornia v. Eli Lilly & Co.,* 43 USPQZd 1938, the Court of Appeals for the Federal Circuit has held that “A written description of an invention

involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, (or) chemical name,' of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

The recitation of “non-standard amino acid degrading” and “glutamate dehydrogenase” fails to provide a sufficient description of the claimed genus of proteins as it merely describes the functional features of the genus without providing any definition of the structural features of the species within the genus. The CAFC in UC California v. Eli Lilly, (43 USPQ2d 1398) stated that: “in claims to genetic material, however a generic statement such as ‘vertebrate insulin cDNA’ or ‘mammalian insulin cDNA,’ without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not

specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus." Similarly with the claimed genus of "non-standard amino acid degrading" and "glutamate dehydrogenase" proteins, the functional definition of the genus does not provide any structural information commonly possessed by members of the genus which distinguish the protein species within the genus from other proteins such that one can visualize or recognize the identity of the members of the genus.

Therefore, in the instant case, the claim is drawn to a method of using a genus of glutamate dehydrogenase having non-standard amino acid degrading activity, but having unknown structure. The specification only describes a method of reducing the incorporation of non-standard amino acids of a heterologous polypeptide produced by a microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the mutant glutamate dehydrogenase of SEQ ID NO:2 or 4 (mutant of the wild type *E. coli* glutamate dehydrogenase of SEQ ID NO:2, wherein said mutant consists of the K92L substitution) and said mutant has norleucine degrading activity. While MPEP 2163 acknowledges that in certain situations "one species adequately supports a genus," it also acknowledges that "[f]or inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus." In view of the widely variant species encompassed by the genus, this one example is not

enough and does not constitute a representative number of species to describe the whole genus of any or all variants, recombinant and mutants of any or all glutamate dehydrogenases having non-standard amino acid degrading activity, including any or all variants, recombinants and mutants thereof, and there is no evidence on the record of the relationship between the structure of the mutant glutamate dehydrogenase having norleucine degrading activity of SEQ ID NO:2 or 4 and the structure of any or all recombinant, variant and mutant of any or all glutamate dehydrogenases having non-standard amino acid degrading activity. Therefore, the specification fails to describe a representative species of the genus comprising any or all glutamate dehydrogenases having non-standard amino acid degrading activity, including any or all variants, recombinants and mutants thereof.

Given this lack of additional representative species as encompassed by the claims, applicants have failed to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize applicants were in possession of the claimed invention.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants should note that the rejection has been amended in light of the amendment of the claims.

Applicants argue that the claims meet the written description requirement because the claims have been amended to require that the non-standard amino acid degrading protein (NSAADP) consist essentially of a wild-type *E. coli* GDH or an *E. coli* GDH and the specification describes using either the wild-type *E. coli* GDH or the K92L variant of *E. coli* GDH. Examiner respectfully disagrees. The phrase "consist essentially" limits the scope of the NSAADP to (A) wild-type *E. coli* GHD or (B) *E. coli* GDH having a leucine instead of lysine at position 92 and optionally, additional amino acids that do not materially affect the basic and novel characteristic of said GDH of (A) or (B). Contrary to applicant's arguments, *E. coli* GDH having a leucine instead of lysine at position 92 is not limited to only the substitution at position 92 because the term "having" in transitional phrases does not create a presumption that the body of the claim is closed (See MPEP 2111.03). Therefore, while the mutant comprises the recited substitution, the same mutant can comprise any amino acids in any other positions. Thus, the claims are drawn to a method of using a genus of polypeptides having NSAADP activity, but having unknown structure.

Applicants also argue that due to the phrase "consists essentially of", the claims are not drawn to any and all variants of a glutamate dehdyrgenase from *E. coli* comprising a lecuine residue at position 92 and any other amino acids at any other position, but rather the skilled artisan would understand that the claims encompass only wild-type *E. coli* GDH, *E. coli* GDH having only the K92L variation and mutant of wild type *E. coli* GDH having the K92L variation and other variations not affecting the NSAADP activity. Examiner respectfully disagrees. As discussed above, the phrase

"consist essentially" limits the scope of the NSAADP to (A) wild-type *E. coli* GHD or (B) *E. coli* GDH having a leucine instead of lysine at position 92 and optionally, additional amino acids that do not materially affect the basic and novel characteristic of said GDH of (A) or (B). Said phrase does not limit the scope of *E. coli* GDH having the K92L variation since the claims **do not** recite that the *E. coli* GDH "consists essentially" of the K92L variation.

Hence, the rejection is **maintained**.

Claims 1, 8-11, 14-15 and 42 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of reducing the incorporation of non-standard amino acids of a heterologous polypeptide produced by a microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the glutamate dehydrogenase of SEQ ID NO:2 or 4, does not reasonably provide enablement for a method of using any or all polypeptides having non-standard amino acid degrading activity, but having unknown structure The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4)

the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

Claims 1, 8-11, 14-15 and 42 are drawn to a method of reducing the incorporation of non-standard amino acids into a heterologous protein in a microorganism by co-expressing in said microorganism said heterologous protein and a non-standard amino acid degrading protein that consists essentially of an *Escherichia coli* glutamate dehydrogenase having a leucine instead of lysine at the amino acid position that corresponds to amino acid position 92 of the wild-type *E. coli* glutamate dehydrogenase.

The breadth of the claims.

It is noted that MPEP 2111.01 states that "[d]uring examination, the claims must be interpreted as broadly as their terms reasonably allow." In this case, the term "having" in transitional phrases does not create a presumption that the body of the claim is closed (See MPEP 2111.03). Therefore, a mutant of a wild-type *Escherichia coli* glutamate dehydrogenase having a leucine instead of lysine at the amino acid position that corresponds to amino acid position 92 of said wild-type glutamate dehydrogenase, wherein said mutant has non-standard amino acid degrading activity, is not limited to only the substitution at position 92. While the mutant comprises the recited substitution, the same mutant can comprise any amino acids in any other positions. Since the claims encompass a method of using any or all mutants of a glutamate dehydrogenase from *E. coli* comprising a leucine residue at position 92 and any other amino acids at

any other position, the claims are drawn to a method of using any glutamate dehydrogenase having non-standard amino acid degrading activity, but having unknown structure. Therefore, the breadth of these claims is much larger than the scope enabled by the specification.

The scope of the claim is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of a method of using any or all polypeptides having non-standard amino acid degrading activity, but having unknown structure. In the instant case, the specification enables only a method of reducing the incorporation of non-standard amino acids of a heterologous polypeptide produced by a microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the glutamate dehydrogenase of SEQ ID NO:2 or 4.

The state of prior art, the relative skill of those in the art, and predictability or unpredictability of the art.

Since the amino acid sequence of the protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. In addition, the art does not provide any teaching or guidance as to (1) which amino acids within a glutamate dehydrogenase can be modified and which ones are conserved such that one of skill in the art can make the recited polypeptides having norleucine

degrading activity, (2) which segments of SEQ ID NO:2 or 4 are essential for activity, and (3) the general tolerance of glutamate dehydrogenase to structural modifications and the extent of such tolerance. The art clearly teaches that changes in a protein's amino acid sequence to obtain the desired activity without any guidance/knowledge as to which amino acids in a protein are required for that activity is highly unpredictable. At the time of the invention, there was a high level of unpredictability associated with altering a polypeptide sequence with an expectation that the polypeptide will maintain the desired activity. For example, Branden et al. (Introduction to Protein Structure, Garland Publishing Inc., New York, page 247, 1991) teach that (1) protein engineers are frequently surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes, (2) the often surprising results obtained by experiments where single mutations are made reveal how little is known about the rules of protein stability, and (3) the difficulties in designing de novo stable proteins with specific functions.

The amount of direction or guidance presented and the existence of working examples.

The specification discloses a method of reducing the incorporation of non-standard amino acids of a heterologous polypeptide produced by a microorganism by transforming into said microorganism a vector comprising said heterologous polypeptide and the glutamate dehydrogenase of SEQ ID NO:2 or 4. However, the speciation fails to provide any information as to (1) norleucine as the substrate associated with any glutamate dehydrogenase isolated from any source, including variants, mutants and

recombinants thereof, (2) structural elements required in a polypeptide having norleucine degrading activity, or (3) which are the structural elements in a glutamate dehydrogenase that are essential to display norleucine degrading activity. No correlation between structure and function of having norleucine degrading activity has been presented. There is no information or guidance as to which amino acid residues in the polypeptides of SEQ ID NO:2 or 4 can be modified and which ones are to be conserved to create a polypeptide displaying norleucine degrading activity.

The quantity of experimentation required to practice the claimed invention based on the teachings of the specification.

While enzyme isolation techniques, recombinant and mutagenesis techniques were known in the art at the time of the invention, e.g. hybridization or mutagenesis, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions. Furthermore, it is not routine in the art to create variants of polynucleotides encoding polypeptides having the activity recited without any knowledge as to the structural features which would correlate with that activity.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method of using any or all polypeptides having non-standard amino acid degrading activity, including variants, mutants, recombinants and fragments thereof. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of any or all mutants, variants and recombinants of any or all polypeptides having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office Action, applicants have traversed the above rejection. Applicants should note that the rejection has been amended in light of the amendment of the claims.

Applicants argue that the claims meet the enablement requirement because the claims have been amended to require that the non-standard amino acid degrading protein (NSAADP) consist essentially of a wild-type *E. coli* GDH or an *E. coli* GDH and the specification describes using either the wild-type *E. coli* GDH or the K92L variant of *E. coli* GDH. Examiner respectfully disagrees. The phrase “consist essentially” limits the scope of the NSAADP to (A) wild-type *E. coli* GHD or (B) *E. coli* GDH having a leucine instead of lysine at position 92 and optionally, additional amino acids that do not materially affect the basic and novel characteristic of said GDH of (A) or (B). However,

E. coli GDH having a leucine instead of lysine at position 92 is not limited to only the substitution at position 92 because the term "having" in transitional phrases does not create a presumption that the body of the claim is closed (See MPEP 2111.03). Therefore, while the mutant comprises the recited substitution, the same mutant can comprise any amino acids in any other positions. Thus, the claims are drawn to a method of using a genus of polypeptides having NSAADP activity, but having unknown structure.

Applicants also argue that due to the phrase “consists essentially of”, the claims are not drawn to any and all variants of a glutamate dehdyrgenase from E. coli comprising a lecuine residue at position 92 and any other amino acids at any other position, but rather the skilled artisan would understand that the claims encompass only wild-type *E. coli* GDH, *E. coli* GDH having only the K92L variation and mutant of wild type *E. coli* GDH having the K92L variation and other variations not affecting the NSAADP activity. Examiner respectfully disagrees. As discussed above, the phrase “consist essentially” limits the scope of the NSAADP to (A) wild-type *E. coli* GHD or (B) *E. coli* GDH having a leucine instead of lysine at position 92 and optionally, additional amino acids that do not materially affect the basic and novel characteristic of said GDH (A) or (B). Said phrase does not limit the scope of *E. coli* GDH having the K92L variation since the claims **do not** recite that the *E. coli* GDH "consists essentially” of the K92L variation.

Hence, the rejection is **maintained**.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 8-11, 14-15 and 42 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Bogosian et al. and Wang et al.

Claims 1, 8-11, 14-15 and 42 are drawn to a method of reducing the incorporation of norleucine into a bovine somatotropin in a microorganism by co-expressing in said microorganism said somatotropin and an *E. coli* glutamate dehydrogenase having a leucine instead of lysine at the amino acid position that corresponds to amino acid position 92 of said wild-type glutamate dehydrogenase.

It is noted that MPEP 2111.01 states that "[d]uring examination, the claims must be interpreted as broadly as their terms reasonably allow." In this case, the term "having" in transitional phrases does not create a presumption that the body of the claim is closed (See MPEP 2111.03). Therefore, a mutant of a wild-type *Escherichia coli* glutamate dehydrogenase having a leucine instead of lysine at the amino acid position that corresponds to amino acid position 92 of said wild-type glutamate dehydrogenase, wherein said mutant has non-standard amino acid degrading activity, is not limited to only the substitution at position 92. While the mutant comprises the recited substitution, the same mutant can comprise any amino acids in any other positions.

Bogosian et al. (JBC, Vo. 264, No. 1, pp. 531-539, 1989 - form PTO-1449) discloses incorporation of norleucine into bovine somatotropin when said bovine somatotropin is expressed in *E. coli* (abstract). Bogosian et al. discusses several options for reducing the incorporation of norleucine (pages 531-532). Bogosian et al. discloses supplementing media with methionine in order to avoid norleucine incorporation into a target protein (page 539) since the additional methionine impedes norleucine from attaching to the $tRNA^{Met}$ (norleucine is charged onto $tRNA^{Met}$ and subsequently incorporated into the protein).

Wang et al. (Eur J Biochem. 2001 Nov;268(22):5791-9 – cited previously on form PTO-892) discloses a mutant glutamate dehydrogenase isolated from *Clostridium symbiosum*, wherein said mutant has a K89L mutation and said mutant has increased activity for degrading norleucine (abstract, Table 4 on page 5796). Lysine at position 89 of *C. symbiosum* glutamate dehydrogenase corresponds to lysine at position 92 of *E.*

coli glutamate dehydrogenase, evidenced by Rice et al. (FEMS Microbiol Rev. 1996 May;18(2-3):105-17 – cited previously on form PTO-892) which discloses a an alignment of *E. coli* glutamate dehydrogenase against *C. symbiosum* glutamate (Figure 1 on page 107). Since (1) there is no limitation on the structure of the claimed variant except having a leucine at position corresponding to position 92 of *E. coli* GDH and (2) the instant claims are drawn to a product, which may be produced by the recited modification/starting material or not, Examiner takes the position that the polypeptide of Wang et al. reads on the instant claims. Whether the claimed product is obtained from wild-type *E. coli* GDH or obtained from any source (including other wild type proteins), as long as the resulting product has the structural limitations recited in the claims (K92L mutation), the product is still the same and is within the scope of the claimed invention.

With the teaching of Wang et al. at hand, one having ordinary skill would have recognized the advantage of expressing the mutant glutamate dehydrogenase of Wang et al. in order to directly degrade norleucine.

Therefore, combining the teachings of Bogosian et al. and Wang et al., it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to reduce the incorporation of norleucine in production of heterologous proteins, such as bovine somatotropin, in *E. coli*, by transforming *E. coli* with a vector comprising said heterologous protein or bovine somatropin and the mutant of Wang et al. or two vectors comprising each of the proteins and supplement the media with methionine to ensure enough availability of methionine since the mutant of Wang et al. also degrades methionine.

One of ordinary skill in the art would have been motivated to combine the above references in order to reduce incorporation of norleucine when producing heterologous proteins in *E. coli* and thereby produce heterologous proteins of interest with minimal norleucine contamination. One of ordinary skill in the art would have had a reasonable expectation of success since Wang et al. teaches a mutant enzyme which degrades norleucine and Bogosian et al. teaches expression of bovine somatotropin.

Therefore, the above references render claims 1, 8-11, 14-15 and 42 *prima facie* obvious.

In response to the previous Office Action, applicants have traversed the above rejection. Applicants should note that the rejection has been amended in light of the amendment of the claims.

Applicants argue that the claims are not obvious over the above references because the claims have been amended to require that the non-standard amino acid degrading protein (NSAADP) consist essentially of a wild-type *E. coli* GDH or an *E. coli* GDH and neither Bogosian et al. nor Wang et al. describes wild-type *E. coli* GDH or an *E. coli* GDH having the K92L mutation. Examiner respectfully disagrees. The phrase "consist essentially" limits the scope of the NSAADP to (A) wild-type *E. coli* GHD or (B) *E. coli* GDH having a leucine instead of lysine at position 92 and optionally, additional amino acids that do not materially affect the basic and novel characteristic of said GDH of (A) or (B). However, *E. coli* GDH having a leucine instead of lysine at position 92 is not limited to only the substitution at position 92 because the term "having" in transitional phrases does not create a presumption that the body of the claim is closed

(See MPEP 2111.03). Therefore, while the mutant comprises the recited substitution, the same mutant can comprise any amino acids in any other positions. As discussed above, since (1) there is no limitation on the structure of the claimed variant except having a leucine at position corresponding to position 92 of *E. coli* GDH and (2) the instant claims are drawn to a product, which may be produced by the recited modification/starting material or not, Examiner takes the position that the polypeptide of Wang et al. reads on the instant claims. Whether the claimed product is obtained from wild-type *E. coli* GDH or obtained from any source (including other wild type proteins), as long as the resulting product has the structural limitations recited in the claims (K92L mutation), the product is still the same and is within the scope of the claimed invention.

Applicants also argue that there is no motivation to add the additional step of co-expressing the GDH of Wang et al. to the method of Bogosian et al. when the skilled artisan would have expected in view of the teachings of Wang et al. that methionine supplementation would still be necessary (to prevent degradation of methionine by the GDH of Wang et al.). Examiner respectfully disagrees. "[I]n considering the disclosure of a reference, it is proper to take into account not only specific teachings of the reference but also the inferences which one skilled in the art would reasonably be expected to draw therefrom." (MPEP 2144.01). Bogosian et al. discloses a method reducing incorporation of norleucine into a heterologous protein indirectly by supplementing media with methionine, thereby impeding norleucine from attaching to the $tRNA^{Met}$ (norleucine is charged onto $tRNA^{Met}$ and subsequently incorporated into the protein). However, with advances in recombinant technology, a mutant glutamate

having increased activity for degrading norleucine was made available (Wang et al. – see abstract, Table 4 on page 5796). With the teaching of Wang et al. at hand, one having ordinary skill would have recognized the advantage of expressing the mutant glutamate dehydrogenase of Wang et al. in order to directly degrade norleucine. Adding the step of co-expressing the mutant GDH of Wang et al. allows for greater reduction of norleucine incorporation into a heterologous protein of the method of Bogosian et al. by directly degrading any norleucine incorporated into said heterologous protein in addition to impeding norleucine from attaching to the $tRNA^{Met}$.

Applicants also argue that there is no motivation to combine the references because supplementation of the media with amino acids such as methionine would be undesirable. Examiner respectfully disagrees. It appears that applicants are arguing that supplementation with amino acids such as methionine would be “undesirable” for economic reasons and not for any technological reasons since applicants do to provide any reasoning/evidence for the latter. The additional expense associated with the addition of amino acids such as methionine would not discourage one of ordinary skill in the art from seeking a superior product, a heterologous protein with reduced contamination with norleucine. See MPEP 2145.

Applicants also argue that the claims are not obvious over the combined teachings of Bogosian et al. and Wang et al. because the instant invention is a simpler process that does not require amino acid supplementation. Examiner respectfully disagrees. The claims do not recite such a limitation. Although the claims are

interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Applicants also argue that there is no reasonable expectation of success that combining Bogosian et al. and Wang et al. would lead to the identification of an alternative to the process of Bogosian et al. which requires amino acid supplementation since the skilled artisan would have recognized that supplementation of the media with amino acids such as methionine was undesirable. Examiner respectfully disagrees. As discussed above, it appears that applicants are arguing that supplementation with amino acids such as methionine would be "undesirable" for economic reasons and not for any technological reasons since applicants do to provide any reasoning/evidence for the latter. The additional expense associated with the addition of amino acids such as methionine would not discourage one of ordinary skill in the art from seeking a superior product, a heterologous protein with reduced contamination with norleucine. See MPEP 2145. Further, the claims do not recite a limitation that the method specifically lacks supplementation with amino acids such as methionine.

Applicants argue on page 16 of the Remarks that the skilled artisan would not have expected that in the absence of any methionine supplementation, co-expression of the GDH mutants of Wang et la. With a heterologous protein would lead to reduction in incorporation of norleucine into the heterologous protein, as seen by 30-fold reduction in the heterologous protein co-expressed with *E. coli* K92L GDH mutant. Examiner respectfully disagrees. The claims do not recite a limitation that the method lacks supplementation with amino acids such as methionine.

Applicants also on pages 16-17 that since the triple mutant of Wang et al. has methionine degrading activity, the skilled artisan would have expected such activity to have an adverse effect on the expression of a heterologous protein and would not have had a reasonable expectation of success in identifying a method that does not require amino acid supplementation. Examiner respectfully disagrees. As discussed above, the claims do not recite a limitation that the method lacks supplementation with amino acids such as methionine. Further, in order to prevent degradation of methionine residues by the action of the mutant of Wang et al., one having ordinary skill in the art would have recognized to add methionine into the media. This addition of methionine has two effects: ensures enough availability of methionine in production of the target protein and impeding norleucine from attaching to the $tRNA^{Met}$ and thereby reducing norleucine incorporation into the protein.

Hence the rejection is **maintained**.

Claims 4-5 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Bogosian et al. and Wang et al. as applied to claims 1, 8-11, 14-15 and 42 above, and further in view of Rice et al.

Claims 4-5 are drawn to a method of reducing the incorporation of non-standard amino acids into a bovine somatropin in a microorganism by co-expressing in said microorganism said somatropin and a non-standard amino acid degrading protein/glutamate dehydrogenase variant (K92L) comprising the amino acid sequence of ID NO:4 which is encoded by a sequence of SEQ ID NO: 3.

As discussed, above, it would have been obvious to one having ordinary skill in the art to reduce the incorporation of non-standard amino acid, norleucine, into a bovine somatotropin in a microorganism by co-expressing in said microorganism said somatotropin and a norleucine degrading mutant of Wang et al. Further, Wang et al. teaches that lysine 89 (which corresponds to lysine 92 in *E. coli* glutamate dehydrogenase) is in the substrate binding site (page 5792). Even though the triple mutant of Wang et al. (K89L/S380A/A163G) no longer recognizes glutamate, said mutant degrades methionine in addition to norleucine. With the teaching at hand, one having ordinary skill in the art would have concluded to either supplement the reaction medium with methionine to ensure sufficient availability of methionine for the production of the target protein or make similar mutations (making substitutions corresponding to residues 89, 380, and 163 of the *C. symbiosum)* in homologous glutamate dehydrogenase, thereby obtaining a mutant having greater substrate specificity towards norleucine by making substitutions corresponding to residues 89, 380, and 163 of the *C. symbiosum*.

Rice et al. (FEMS Microbiol Rev. 1996 May;18(2-3):105-17 - form PTO-892) discloses a an alignment of three glutamate dehydrogenase against *C. symbiosum* glutamate dehydrogenase, including glutamate dehydrogenase isolated from *E. coli* (Figure 1 on page 107). Lysine at position 89, serine at position 380 and alanine at position 163 of *C. symbiosum* glutamate dehydrogenase corresponds to lysine at position 89, serine at position 380 and alanine at position 163 of *E. coli* glutamate dehydrogenase.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to make K89L/S380A/A163G mutations in an *E. coli* glutamate dehydrogenase in order to make an enzyme that has greater substrate specificity towards norleucine over other natural and non-standard amino acids and use said mutant enzyme to reduce incorporation of norleucine in heterologous proteins in *E. coli*.

One of ordinary skill in the art would have been motivated to make the above mutant in order to obtain an enzyme having greater substrate specificity towards norleucine thereby providing a norleucine degrading enzyme available for use in reducing incorporation of norleucine into a target protein. One of ordinary skill in the art would have had a reasonable expectation of success since lysine 92(E. coli)/lysine 89 (*C. symbiosum*) is in the substrate binding pocket and making site specific mutations are routine.

Therefore, the above references render claims 4-5 *prima facie* obvious.

In response to the previous Office Action, applicants have traversed the above rejection.

Applicants argue that for the reasons explained in the preceding section (pages 10-17 of the Remarks), the skilled artisan would not have had a reason to combine Bogosian et al. and Wang et al., the skilled artisan also would not have had a reasonable expectation that co-expressing a mutant GDH of Wang et al. with a heterologous protein would yield an alternative means for reducing the incorporation of norleucine into the heterologous protein which did not require supplementation of the

media with methionine, expression of *E. coli* K92L GDH unexpectedly reduced the percentage of bST containing norleucine by nearly 30 fold in the absence of any amino acid supplementation, and the triple mutant of Wang et al. clearly exhibits methionine degrading activity. See the rebuttal above.

Hence the rejection is **maintained**.

Conclusion

Claims 1, 4-5, 8-11, 14-15 and 42 are rejected.

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yong Pak whose telephone number is 571-272-0935. The examiner can normally be reached 6:30 A.M. to 5:00 P.M. Monday through Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

/Yong D Pak/
Primary Examiner, Art Unit 1652